Hacquetia 22/1 • 2023, 81–90 DOI: 10.2478/hacq-2022-0015 sciendo

Phenotypic diversity of natural populations of an endemic Moroccan plant (*Euphorbia resinifera* O. Berg)

Hassane Abd-dada[1], Said Bouda[1], Youssef Aitbella[1] & Abdelmajid Haddioui[1]

Key words: *Euphorbia resinifera*, Endemic plant, Morocco, Morphological variability.

Ključne besede: *Euphorbia resinifera*, endemična rastlina, Maroko, morfološka variabilnost.

Abstract

The *Euphorbia resinifera* is melliferous and medicinal plant and one of the endemic species of Moroccan Atlas Mountains. It is very well known for its honey of high nutritional and therapeutic quality. The objective of our study is to characterize and evaluate the phenotypic variation of this spontaneous species. Seventeen qualitative and quantitative morphological characters related to the bush, stem, spine, flower, and fruit of the plant were used to assess the morphological variability of twelve natural populations collected from its geographical range in Morocco. The results of the analysis of variance showed significant differences between the studied populations for the most examined traits reflecting the existence of a high phenotypic variability within this species. The principal component analysis showed that *E. resinifera* populations were clustered in three distinct groups not related to mountain range type. The results highlight a high phenotypic diversity within studied populations of this species in Morocco. This work aims to study the phenotypic variability of *E. resinifera* to delineate conservation strategies and also to establish forms of rational economic exploitation.

Izvleček

Euphorbia resinifera je medonosna in zdravilna rastlina in eden od endemitov gorovja Atlas v Maroku. Njen med je znan po visoki hranilni vrednosti in terapevtski kakovosti. Cilj raziskave je ugotoviti in oceniti fenotipsko variabilnost te vrste. Za analizo morfološke variabilnosti dvanajstih naravnih populacij s širšega geografskega področja v Maroku smo uporabili sedemnajst kvalitativnih in kvantitativnih morfoloških znakov, povezanih z grmiščnim habitusom, steblom, bodicami, cvetom in plodom. Rezultati analize variance so pokazali značilne razlike med obravnavanimi populacijami za večino znakov in veliko fenotipsko variabilnost znotraj vrste. Z metodo glavnih komponent smo pokazali, da se populacije vrste *E. resinifera* združujejo v tri skupine, ne glede na lokacijo gorovja. Rezultati potrjujejo veliko fenotipsko raznolikost med populacijami te vrste v Maroku. Z raziskavo fenotipske plastičnosti želimo začrtati strategije ohranjanja in tudi vzpostaviti oblike racionalnega gospodarskega izkoriščanja.

Corresponding author:
Abdelmajid Haddioui
E-mail: ahaddioui@yahoo.fr

Received: 9. 2. 2021
Accepted: 11. 12. 2022

1 Laboratory of agro-industrial and medical Biotechnologies, Faculty of Sciences and Techniques, Sultan Moulay Slimane University, B.P. 523, Beni Mellal, Morocco

Introduction

Euphorbia is considered the second largest genus in the angiosperms and the largest genus of Euphorbiaceae, it has a cosmopolitan distribution with about 2000 species (Bruyns et al., 2011, Fayed et al., 2020). The great diversity present in their growth forms, and many xerophytic taxa were seen in this genus (Talebi et al., 2016). *Euphorbia* species are mostly herbs, some shrubs, but rarely trees (Davis et al., 1994, Dorsey et al., 2013). They are mostly monoecious, although some are dioecious (Prenner & Rudall, 2007).

Euphorbia resinifera is represented worldwide by two varieties namely, *Euphorbia resinifera* var. *chlorosoma* Croizat and *Euphorbia resinifera* var. *typica* O. Berg (Govaerts et al., 2000; Eggli., 2004) which is the only one found in Morocco (Jahandiez & Maire, 1932; Fennane et al., 2007). *Euphorbia resinifera* var. *typica,* locally named "zeggoum" is native and endemic to Morocco (Jahandiez & Maire, 1932) and is found at the high plateaus of the Atlas Mountains, particularly in Beni Mellal-Khenifra region. The plant extends from El Ksiba to Demnat in a very discontinuous way according to a climatic gradient and covers an area over than 8000 ha MMA, 2018). *E. resinifera* is a dense succulent shrub growing to 1 meter in height, forming dense bushes of 0.5 to 2 meters in diameter (Figure 1). The plant has green, tetragonal, branched, tight and spiny stems (stipular spines). The many small yellow flowers that appear in late spring at the end of the stems attract and feed the bees. The fruit is a small, trilobed, and trilocular capsule each one containing an almost round seed (Fennane & Ibn-Tattou, 2007).

E. resinifera has a considerable importance in the structure and functioning of pre-forest ecosystems, as it contributes, by their juxtaposed stem, to the decrease of soil erosion, and attracts and feeds the bees with their small yellow flowers (Benabid, 2002).As a melliferous plant, it is very well known for its honey of high nutritional and therapeutic quality (Moujanni et al., 2017, Bettar et al., 2019, Ihitassen et al., 2019), which has been recognized as a terroir product of the region and has been labeled Protected Geographical Indication (PGI) (MMA, 2012). Thus, it has a major socio-economic role in the region by creating high added value. Consequently, it is an income-generating activity with nearly 47.000 units of apiaries distributed on 35 cooperatives and an annual honey production of about 300 tons. For this reason, this species must be considered as an important support for local development.

In addition to its melliferous qualities, the species has an enormous number of therapeutic properties. Indeed, the dried latex of this plant, named "euphorbium", was used as a vesicatory, sternutatory and powerful laxative and as a remedy against poisoning and snake bites (Agrawal & Konno, 2009). The irritant compound resiniferatoxin identified in the latex of this species in 1975 (Hergenhahm et al., 1975) has many potential medical applications (Appendino et al., 2010). The diterpenic

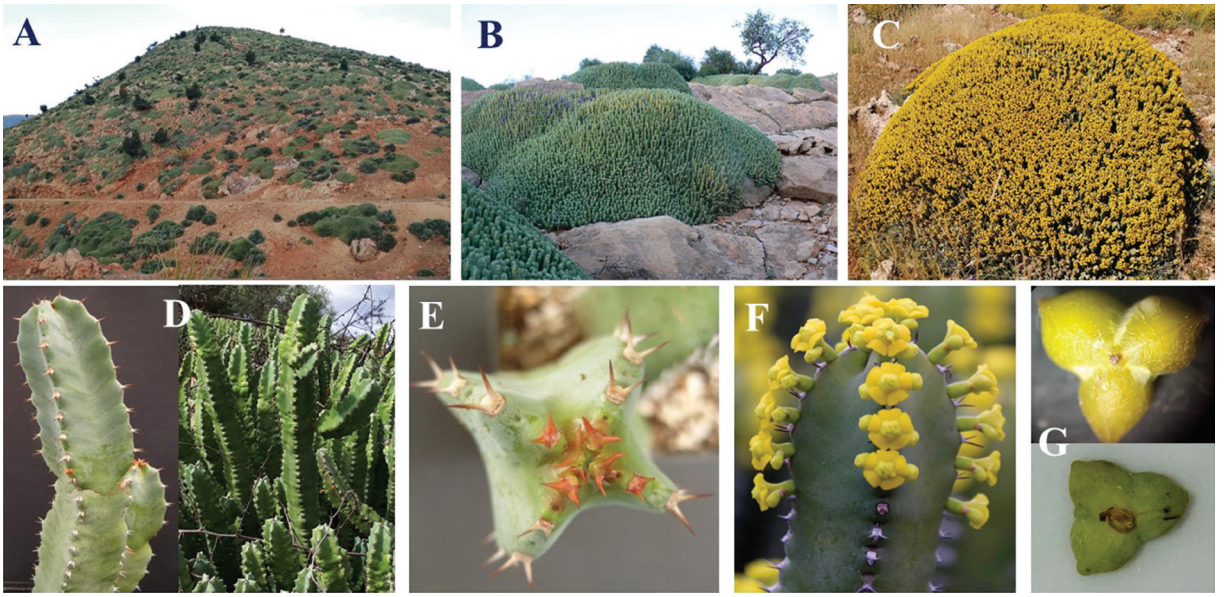

Figure 1: Constituents of studied plant. A – *Euphorbia resinifera* population; B – bush without flowers; C – bush with flowers; D – stems; E – spines in tetragon stem; F – flowers; G – fruit.

Slika 1: Deli preučevane vrste. A – populacija *Euphorbia resinifera*; B – grm brez cvetov; C – grm s cvetovi; D – stebla; E – bodice na štirikotnem steblu; F – cvetovi; G – plod.

compounds of the latex are an anti-pain, anti-tuberculosis (Sharma et al., 2019), antioxidant, antibacterial, antifungal (Farah & Ech-chahad, 2014) antiparasitic (Mazoir et al., 2011), anticancer (Zhang et al., 2019; Talbaoui & Hamdaouic, 2020) and they are used to inhibit the phytotoxicity of some insects (Mazoir et al., 2008). In Morocco, *E. resinifera* is traditionally used to treat: glycaemia in type II diabetics, some types of severe dermatoses, snake bites and for cancer treatment (Bellakhdar et al., 1991; Bourhia et al., 2019).

Although its many benefits, *E. resinifera* is undergoing a great anthropic pressure. Natural fires, clearing by local populations for agricultural and forage, and urban planning purposes, its use for domestic burning as well as the exploitation of stone quarries have largely and significantly contributed to the reduction of its population in the recent years (Nemmaoui et al., 2013).

Thus, emergency measures should be taken to safeguard this wild and endemic species in Morocco. Therefore, it has become imperative to evaluate the diversity of the populations of this species. To the best of our knowledge, no data deal with the variability is available for this species. The obtained information would make it possible to develop strategies to conserve and improve the local *E. resinifera* genotypes.

Traditionally, the amplitude and distribution of plant genetic diversity has been widely assessed based on conventional morphological traits. Despite the fact that the expression of these traits is strongly affected by environmental conditions of the species, they are highly recommended as a first step that should be achieved before biochemical, molecular and technological analysis. In this context, the present study aimed to characterize and evaluate the diversity of natural populations of this endemic species using morphological traits. Therefore, the establishment of these traits is necessary for the characterization and identification of local populations for a better scientific understanding of this species.

Materials and Methods

During 2019, 12 natural populations of *E. resinifera* throughout its geographical range in Morocco (Figure 2) were sampled on the basis of the difference in their geographical distances and altitude. Geographic characteristics such as altitude, latitude, and longitude as well as the mean precipitation of these populations are summarized in Table 1. For each population, 10 bushes were randomly chosen and sampled. From each bush, 10 stems, 10 spines and 10 fruits per stem were studied. The analysis of morphological variations was based on 17 qualitative and quantitative traits related to bush, stem, spine, flower, and fruit (Table 2).

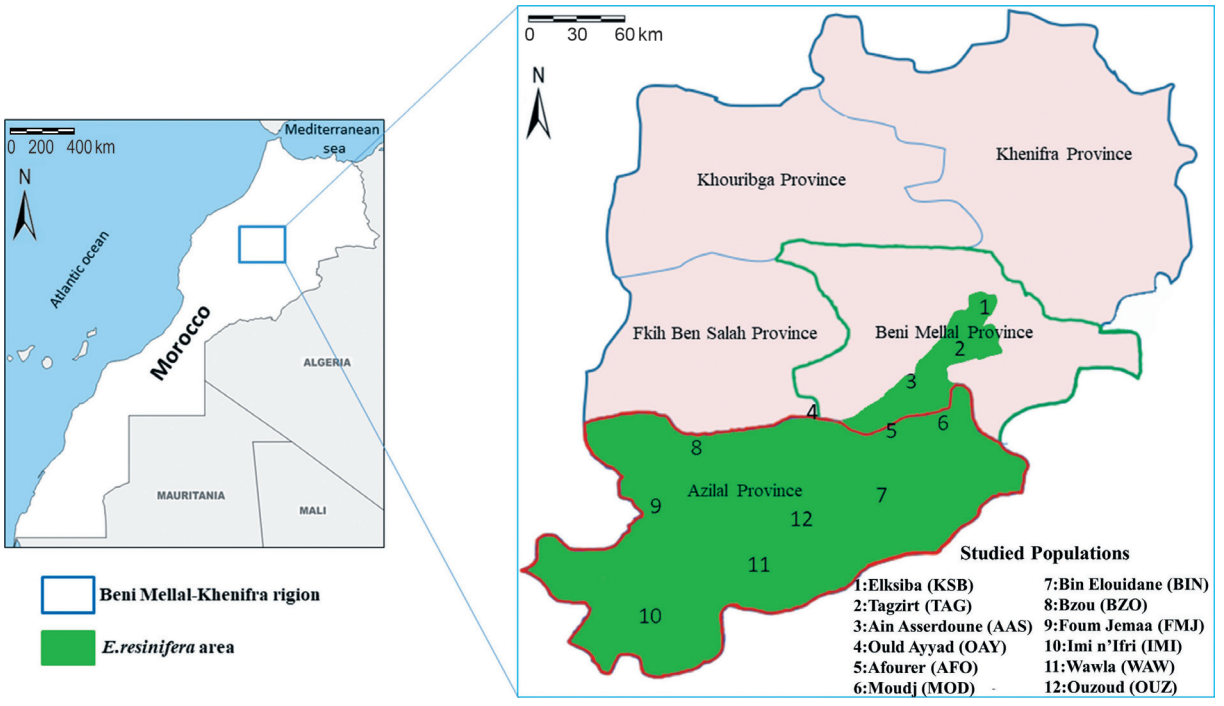

Figure 2: Map of Morocco showing locations of the *Euphorbia resinifera* populations studied (Ettaki et al., 2020).
Slika 2: Zemljevid Maroka z lokacijami preučevanih populacij *Euphorbia resinifera* (Ettaki et al., 2020).

Table 1: Geographical and meteorological conditions of *Euphorbia resinifera* natural populations used in the study.
Tabela 1: Geografske in meteorološke razmere naravnih populacij vrste *Euphorbia resinifera* v raziskavi.

Populations	Code	Geographic origin	Zone	Altitude (m)	Latitude N	Longitude W	Rainfall average (mm/yr)	Temperature average (°C/yr)
Elksiba	KSB	1 km N of Elsiba	Middle Atlas	1045	32°34'	6°2'	718	16.4
Tagzirt	TAG	5 km Northeast of Taghzirt	Middle Atlas	751	32°25'	6°11'	550	18.5
Ain Asserdoune	AAS	2 km South of Beni Mellal	Middle Atlas	771	32°19'	6°19'	493	18.3
Oulad Ayyad	OAY	4 km South of Oulad Ayyad	Middle Atlas	564	32°11'	6°48'	506	18.9
Afourer	AFO	6 km South of Afourer	Middle Atlas	793	32°11'	6°31'	443	18.8
Modj	MOD	15 km East of Beni Mellal	Middle Atlas	1223	32°17'	6°18'	550	18.1
Bin El Ouidane	BIN	6 km Southwest of Bin El Ouidane	High Atlas	936	32°4'	6°27'	490	17.6
Bzou	BZO	3 km South of Bzou	High Atlas	494	32°5'	7°3'	350	19.3
Foum Jemaa	FMJ	7 km West of Foum Jemaa	High Atlas	773	31°59'	7°1'	444	17.4
Imi n'Ifri	IMI	10 km East of Demnat	High Atlas	1127	31°43'	6°58'	478	16.4
Wawla	WAW	30 km East of Azilal	High Atlas	1237	31°54'	6°43'	521	16.2
Ouzoud	OUZ	3 km North of Ouzoud	High Atlas	941	32°10'	6°41'	456	17.7

Table 2: Morphological traits analyzed of Moroccan natural populations of *Euphorbia resinifera.*
Tabela 2: Morfološki znaki obravnavanih naravnih populacij vrste *Euphorbia resinifera* v Maroku.

Bush Traits	Label
Diameter of the bush (cm)	DB
Type of substrate in which the bush is growing	TSBG
Type of stems in the bush	TSB
Presence of branched stems in the bush	PBSB
Stem traits	Label
Height of the stem (Stem length) (cm)	HS
Distance between the dimensions of the tetragon stem (mm)	DBDTS
Color of the stem	CS
Latex presence in the stem	LPS
Spine traits	Label
Length of spines of 10 upper pairs in the tetragon stem (mm)	LSPS
Distance between 10 upper pairs of spines in the tetragon stem (mm)	DPSTS
Color of the spines of each pair	CSP
Flower traits	Label
Length of the flower (mm)	LF
Number of flowers in the stem	NFS
Length of the floral peduncle (mm)	LFP
Fruit traits	Label
Fruit thickness (mm)	FT
Fruit diameter (mm)	FD
Fruit weight (g)	FW

The obtained data was submitted to one-way analysis of variance (ANOVA) to detect significant differences between the studied populations for the parameters analyzed. Correlations between traits were determined using the Pearson correlation coefficient. Also, relationships among the populations were investigated using principal components analysis (PCA) and hierarchical cluster analysis using respectively the statistical software XLSTAT® (version 2020.1) and Statistica (version 10, StatSoft).

Results

The results of analysis of variance of qualitative traits showed highly significant differences between studied populations for the color of the stem and latex presence in the stem (Table 3). The observation data showed that many plants of this species have shown bushes growing on the rock (TSBG) (72.5%). However, 27.5% of the plants have shown bushes growing on the soil. Also, the data obtained showed that half of plants have tight stems (TSB) (50.83%) followed by medium stems (29.6%) and open stems (20%). Moreover, 68.33% of the bushes contain branched stems (PBSB) and 31.67% have unbranched stems. Among the three stem color (CS) observed, the normal green color (86.66%) was the most dominant. Latex is very present in all the populations (LPS) (90% of the total plants). The rest varies between medium (8.34%) and low presence of the latex (1.66%). Finally, the color of the spines (CSP) was predominantly white-brown (70.05%) followed by red color (29.95%).

The average values for all quantitative parameters measured are presented in Table 4. Analysis of variance showed highly significant differences between populations for all the morphological characters, except the diameter of the bush. This result showed high degree of diversity in the Moroccan *E. resinifera* populations. The morphological parameters that had the greatest variability

Table 3: Dominant frequencies and analysis of variance of qualitative traits.
Tabela 3: Dominantne frekvence in analiza variance kvalitativnih znakov.

Trait	Evaluation scale	Dominant character	Frequency %	CV %
TSBG	Soil – Rock	Rock	72.5 ^{ns}	10.51
TSB	Tight – Medium – Open	Tight	50.83 ^{ns}	24.38
PBSB	Yes – No	Yes	68.33 ^{ns}	22
CS	Pale green – Normal green – Dark green	Normal green	86.66 **	12.06
LPS	Low – Medium – Very present	Very present	90 **	7.34
CSP	Red – White-brown	White-brown	70.05 ^{ns}	0

ns: not significant; **: significant at the 1% probability level; CV: Coefficient of variation.
Abbreviations as in table 2.

Table 4: Mean and 'F' value from one-way ANOVA of morphological quantitative traits.
Tabela 4: Povprečja in 'F' vrednosti iz eno-faktorske ANOVA morfoloških kvantitativnih znakov.

Populations	DB	HS	DBDTS	LSPS	DPSTS	LF	NFS	LFP	FT	FD	FW
Elksiba (KSB)	**83**	48.43	32.04	**1.95**	**2.77**	**7.50**	12.92	**4.49**	5.22	6.75	0.10
Tagzirt (TAG)	168.3	59.93	31.95	3.48	4.11	12.59	13.61	9.38	4.77	5.68	0.07
Ain asserdoune (AAS)	193.7	**67.01**	**38.39**	2.86	5.39	**14.72**	14.79	11.07	5.69	**7.99**	0.10
Afourer (AFO)	160.2	61.29	31.48	3.19	5.59	11.60	**12.5**	8.37	5.56	7.52	0.10
Oulad Ayyad (OAY)	160.6	57.23	34.96	3.29	4.55	10.28	13.98	7.22	**4.58**	6.59	0.09
Bzou (BZO)	**196.6**	50.70	**28.26**	3.77	4.45	8.57	13.65	5.50	4.78	7.16	**0.05**
Foum Jemaa (FMJ)	169.9	51.10	32.95	3.40	4.96	14.66	14.06	**11.08**	5.22	7.89	0.10
Imi n'Ifri (IMI)	136.2	48.46	34.92	3.51	4.54	9.78	14.11	6.84	**5.82**	7.69	**0.17**
Wawla (WAW)	154.3	**46.42**	30.68	**3.88**	3.06	12.56	**14.99**	9.37	4.61	**5.36**	0.06
Ouzoud (OUZ)	157.5	49.02	33.81	3.63	4.98	13.30	14.37	9.14	5.21	6.56	0.09
Bin El Ouidane (BIN)	172.7	48.15	36.74	3.12	4.73	13.22	13.82	9.13	4.77	5.59	0.09
Modj (MOD)	155.5	47.14	35.74	3.28	**7.12**	12.96	14.22	8.78	5.17	7.02	**0.05**
Mean	159.04	52.91	33.50	3.28	4.69	11.82	13.92	8.37	5.12	6.82	0.09
CV%	18.33	12.72	8.45	15.36	24.14	19.58	5.06	24.17	8.26	13.26	37.27
F value	1.76	2.72**	4.07***	4.48***	6.04***	23.01***	1.92*	19.79***	66.98***	109.6***	1.91*

Significance level:
***: significant at the 0.1% probability level; **: significant at the 1% probability level; *: significant at the 5% probability level.
The minimum and maximum are written in Bold; CV: Coefficient of variation

were in general those related to the height of stem (HS), length of spines of 10 upper pairs in the tetragon stem (LSPS), distance between 10 upper pairs of spines in the tetragon stem (DPSTS), length of the flower (LF), length of the floral peduncle (LFP), fruit diameter (FD) and fruit weight (FW). The data showed also that the highest stem (HS) was found in populations Ain Asserdoune (67.01cm), while the lowest value was recorded in Wawla population (46.42 cm). The data obtained reveal that the population Wawla had the highest length spines (LSPS) (3.88 mm) and El Ksiba the lowest (1.95 mm). In addition, the distance between 10 upper pairs of spines in the stem (DPSTS) ranged from 2.77 mm in El Ksiba to 7.12 mm in Modj population with general mean of 4.69 mm. Regarding weight fruit, the highest value was found in population Imi n'ifri (0.17 g) and the lowest was observed in Bzou and Modj populations (0.05g). The fruit diameter varied from 5.36 mm for Wawla population to 7.99 mm for Ain Asserdoune population with a general average of 6.82 mm. The fruit thickness ranged from 4.58 mm to 5.82 mm. The greatest thickness was observed in Imi n'ifri population whereas the lowest was registered in Oulad Ayyad population. Also, the diameter of the bushes oscillated between 83 cm for El Ksiba to 196.6 cm for Bzou populations with general average of 159.04 cm. The distance between the dimensions of the tetragon stem ranged from 28.26 mm to 38.39 mm. Maximum distance was found in Ain Asserdoune population while minimum distance was observed in Bzou population. Further, the number of the flowers in the stem varied ranged from 12.5 for Afourer to 14.99 for Wawla population with a general average of 13.92.

To highlight the strength and direction of association between the morphological characters, Pearson correlation analysis was carried out (Table 5). Significant correlations were found between some morphological characters. Length of the flower (LF) had positive and significant correlation with length of the floral peduncle (LFP, r = 0.98). Also, fruit diameter (FD) was positively and significantly correlated with fruit thickness (FT, r = 0.71). Further, height of the stem (HS) was positively correlated with distance between the dimensions of the tetragon stem (DBDTS, r = 0.59). In addition, the diameter of the bush (DB) was positively correlated with type of stems in the bush (TSB, r = 0.71), height of the stem (HS, r = 0.66), distance between the dimensions of the tetragon stem (DBDTS, r = 0.5). In contrast, height of the stem (HS) was negatively correlated with type of substrate in which the bush is growing (TSBG, r = -0.43) and presence of branched stems in the bush (PBSB, r = -0.56). Also, type of substrate in which the bush is growing (TSBG) was found to be negatively correlated with type of stems in the bush (TSB, r = -0.57). On the other hand, all morphological characters and geographic parameters show a low significant correlation, except the traits length of spines of 10 upper pairs in the tetragon stem with latitude (LSPS, r = -0.40), longitude (r = 0.39) and precipitation (r = -0.41), and latex presence in the stem with precipitation (LPS, r = -0.33).

Principal component analysis (PCA) was performed considering all parameters measured. The eigenvalues obtained by PCA indicate that the first two components provide a good summary of the data. They explained 52.63% of the total variability. The first component, PC1, which represents variables related to diameter of the bush (DB), type of stems in the bush (TSB), latex presence in the stem (LPS), length of the flower (LF), length of the floral peduncle (LFP), and presence of branched stems in the bush (PBSB) accounted for about 33.15% of total variation. PC2 which explained variables associated with distance between the dimensions of the stem (DBDTS), fruit diameter (FD), fruit thickness (FT) and fruit weight (FW), comprised about 19.49% of the variation. The plot for the first two principal components showed a high dispersion of studied populations. Three groups of populations were differentiated (Figure 3). The first cluster was constituted with eight populations, five from High Atlas Mountain (Foum Jemaa, Ouzoud, Bin El Ouidane, Bzou and Wawla) and three from Middle Atlas Mountain (Modj, Oulad Ayyad and Tagzirt). These populations are characterized by bushes with diameters between 1,5 and 2 meters, stem height that varies between 46 and 60 cm, long spines, high number of flowers in the stems and relatively low fruit weights. The second group contains three populations, one from the High Atlas Mountain (Imi n'Ifri) and two from Middle Atlas Mountain (Ain Asserdoune and Afourer). This group is distinguished by diameter of the bushes varying between 1,3 and 2 meters, stem height comprised between 48 and 67 cm, and relatively high fruit weights. The third group contained one population from the Middle Atlas Mountain (Elksiba), differentiated from other group by bushes less than 1 meter in diameter, stems often very tight and unbranched, short spines and small flowers. Then, the grouping of the populations in three groups was according to morphological traits and not to the mountain range type.

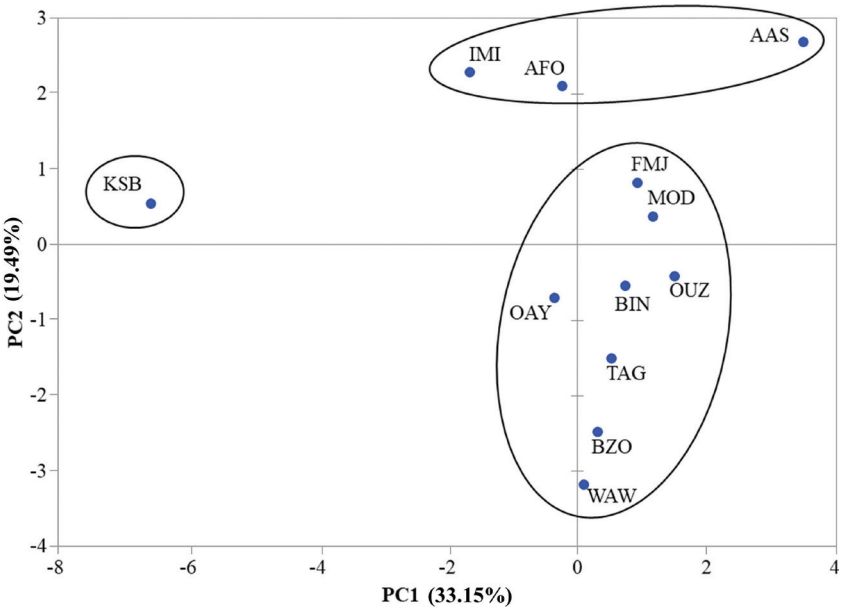

Figure 3: Principal component analysis (PCA) score plot of 12 natural populations of *Euphorbia resinifera* based on the first two principal components (KSB: Elksiba; TAG: Tagzirt; AAS: Ain Asserdoune; OAY: Ould Ayyad; AFO: Afourer; MOD: Moudj; BIN: Bin Elouidane; BZO: Bzou; FMJ: Foum Jemaa; IMI: Imi n'Ifri; WAW: Wawla; OUZ: Ouzoud).

Slika 3: Graf metode glavnih komponent (PCA) 12 naravnih populacij *Euphorbia resinifera* prvih dveh glavnih komponent (KSB: Elksiba; TAG: Tagzirt; AAS: Ain Asserdoune; OAY: Ould Ayyad; AFO: Afourer; MOD: Moudj; BIN: Bin Elouidane; BZO: Bzou; FMJ: Foum Jemaa; IMI: Imi n'Ifri; WAW: Wawla; OUZ: Ouzoud).

Table 5: Matrix of correlation between geographic parameters, rainfall and morphological characters measured of 12 natural populations of *Euphorbia resinifera* in Morocco.
Tabela 5: Korelacijska matrika med geografskimi dejavniki, padavinami in morfološkimi značilnostmi iz 12 naravnih populacij vrste *Euphorbia resinifera* v Maroku.

	DB	TSBG	TSB	PBSB	HS	DBDTS	CS	LPS	LSPS	DPSTS	LF	NFS	LFP	FT	FD	FW	Altitude	Latitude	Longitude	Precipitation (mm)
DB																				
TSBG	-0.44***																			
TSB	0.71***	-0.57***																		
PBSB	-0.047***	0.26**	-0.41***																	
HS	0.66***	-0.43***	0.58***	-0.56***																
DBDTS	0.5***	-0.24**	0.44***	-0.4***	0.59***															
CS	0.10	0.05	0.03	-0.1	0.36***	0.19*														
LPS	0.16	-0.14	0.13	-0.16	0.10	0.18	0.37***													
LSPS	0.27**	-0.34***	0.28**	-0.24**	0.17	0.11	0.07	0.23*												
DPSTS	0.25**	-0.13	0.25**	-0.07	0.11	0.25**	-0.02	0.15	0.19*											
LF	0.29**	-0.15	0.27**	-0.25**	0.12*	0.32***	0.15	0.23*	0.26**	0.38***										
NFS	0.4***	-0.21*	0.28**	-0.28**	0.31**	0.45***	0.14	0.18*	0.25**	-0.01	0.26**									
LFP	0.29**	-0.14	0.26**	-0.27**	0.24**	0.3**	0.19*	0.22*	0.27**	0.32***	0.98***	0.26**								
FT	0.02	-0.04	0.07	0.16	0.14	0.22*	-0.06	-0.06	-0.15	0.25**	0.09	-0.05	0.1							
FD	0.02	0.01	0.04	0.02	0.13	0.10	-0.04	0.07	-0.09	0.32**	0.02	-0.03	0.03	0.71**						
FW	-0.03	-0.12	0.1	-0.01	0.1	0.17	0.03	0.06	-0.01	-0.03	-0.01	0.10	0.04	0.23*	0.18					
Altitude	-0.21*	0.08	-0.10	0.14	-0.27**	0.11	-0.12	-0.05	-0.05	-0.01	0.09	0.12	0.05	0.21*	-0.22*	0.06				
Latitude	-0.09	0.02	-0.03	0.03	0.19*	0.02	0.14	-0.27**	-0.4***	-0.02	-0.12	-0.17	-0.14	-0.1	-0.08	-0.15	-0,18*			
Longitude	0.15	-0.02	0.05	-0.1	-0.11	-0.15	-0.14	0.24**	0.39***	0.02	-0.03	0.10	0.01	-0.05	0.27**	0.07	-0,29**	-0,83***		
Precipitation (mm)	-0.31**	0.15	-0.25*	0.13	-0.06	0.1	-0.01	-0.33***	-0.41***	-0.23*	-0.21*	-0.06	-0.21*	-0.01	-0.27**	0.01	0,49***	0,62***	-0,78***	

Significance level:

***: significant at the 0.1% probability level; **: significant at the 1% probability level; *: significant at the 5% probability level.

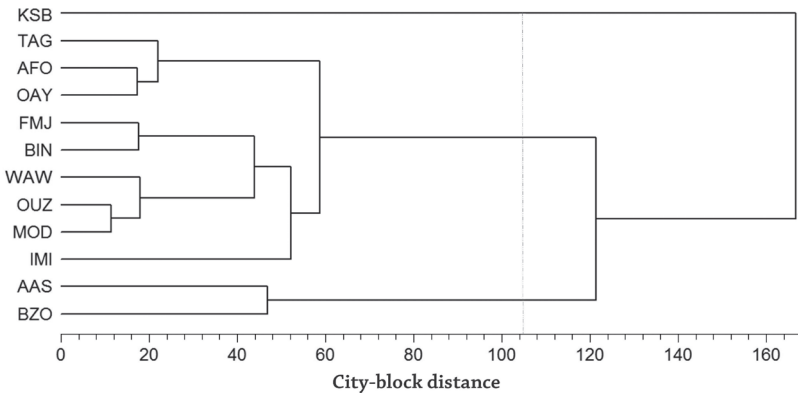

Figure 4: UPGMA cluster analysis of the studied *Euphorbia resinifera* populations based on morphological traits (KSB: Elksiba; TAG: Tagzirt; AAS:Ain Asserdoune; OAY: Ould Ayyad; AFO: Afourer; MOD: Moudj; BIN: Bin Elouidane; BZO: Bzou; FMJ: Foum Jemaa; IMI: Imi n'Ifri; WAW: Wawla; OUZ: Ouzoud).
Slika 4: Klastrska analiza UPGMA preučevanih populacij *Euphorbia resinifera* z morfološkimi znaki (KSB: Elksiba; TAG: Tagzirt; AAS: Ain Asserdoune; OAY: Ould Ayyad; AFO: Afourer; MOD: Moudj; BIN: Bin Elouidane; BZO: Bzou; FMJ: Foum Jemaa; IMI: Imi n'Ifri; WAW: Wawla; OUZ: Ouzoud).

The cluster analysis of the studied populations based on 17 traits led to the identification of three groups (Figure 4). The first cluster comprised two populations: one originating from Middle Atlas Mountain (Ain Asserdoune) and another originating from High Atlas Mountain (Bzou). The second group bifurcated in two subgroups; the first subgroup contains five populations belonging to the High Atlas Mountain (Foum Jemaa, Bin El ouidane, Wawla, Ouzoud and Imi n'Ifri) and one taking its rise in the Middle Atlas Mountain (Modj). The second subgroup consisted of three populations (Tagzirt, Afourer and Oulad Ayyed) coming from Middle Atlas Mountain. The Middle Atlas Mountain population's Elksiba formed alone a third group that is highly divergent from the remaining groups. As PCA results, the genetic structure of investigated *Euphorbia* bushes within three main gene pools was not correlated to the mountain range type.

Discussion

Morphological characters are important and have been traditionally used for identification and characterization of plants. They are the first traits to use to study the diversity of species and populations. In this study, we used some qualitative and quantitative traits related to bush, stem, spine, flower, and fruit for examining the phenotypic diversity of twelve Moroccan *E. resinifera* populations. The results obtained show significant differences between the studied populations for the most examined parameters suggesting the existence of a high phenotypic variability within this species.

Regarding the type of substrate in which the bush is growing, the results obtained showed that many plants of this species (72.5%) are growing on the rock and only 27.5% on the soil. According to consulted literature, *E. resinifera* is found on rocky and calcareous substrates (Jahandiez & Maire, 1932). Moreover, 68.33% of the bushes contain branched stems and 31.67% have unbranched stems. About color of spine, our results revealed that white-brown was the predominant color in Moroccan populations of this species.

On the other hand, the data obtained showed that the length of spines ranged from 1.95 mm to 3.88 mm. Moreover, the diameter of the bush varied from 83 cm to 196.6 cm. These results seem to be similar to those obtained by Fennane & Ibn-Tattou (2007) for the same species (50 to 200 cm). In addition, half of the plants have tight stems and almost 30% medium stems. As a result, *E. resinifera* has a cushion-shaped physiognomy consisting of a bush of juxtaposed stems. Their growth in height does not exceed one meter on average, however their lateral growth is much greater by the emission of new lateral stems at the basis of the old ones (diameter of the bush varied between 83 and 196.6 cm). This creeping behavior of this species would have a considerable role against soil erosion. So, the *E. resinifera* species could be used for reforestation and rehabilitation of degraded areas, thus leading to restore the vegetative soil covers and preserves the associated vegetation. Nevertheless, studies on the regeneration of *E. resinifera* are needed.

Regarding the fruit, the fruit weight varied from 0.05 g to 0.17 g and the fruit thickness oscillated between 4.58 mm to 5.82 mm. Furthermore, our result showed that the latex (liquid rich in diterpenic compounds) is very present in almost all populations (90% of the total plants). This result is similar to that obtained for some *Euphorbia* species (Mahlberg et al., 1983).

Moroccan *E. resinifera* populations were clustered in three distinct groups. This finding is different with that reported by Pahlevani et al. (2017) concerning some species of the genus *Euphorbia* (*Euphorbia austro-iranica, E. buhsei, E. khabrica*, and *E. osyridea*). The structuration of the populations was operated according to their morphological traits but independently from mountain range type. This indicates that climatic conditions have had little effect on populations' structuration which implies that there is no local adaptation of studied populations;

and which is strengthened by the no existence of correlation between ecological parameters such as altitude, mean precipitation and mean temperature and phenotypic traits. Consequently, isolation by barriers has not played an important role in establishing the clustering of these natural populations. Similar results were found by Kabiri et al. (2018, 2019) by studying genetic diversity and structure of Moroccan Walnut (*Juglans regia*) populations using successively morphological and ISSR traits. However, the genetic structure of natural populations of Maritime Pine (*Pinus pinaster* Aiton), investigated by isozymes, revealed to be correlated to mountain range type in Morocco (Wahid et al., 2004).

Conclusions

The results presented in this paper are the first report to assess the phenotypic diversity of Moroccan *E. resinifera* populations. These results show that *E. resinifera* which is neglected by researchers until now presents a high variability among populations with regard the plant and fruit traits. This high level of variability can be used in genetic resource conservation programs. Further investigations that would be a complement for this study are currently underway using chemical, biochemical and molecular traits.

Said Bouda https://orcid.org/0000-0001-7208-0255
Abdelmajid Haddioui https://orcid.org/0000-0001-8860-6194

References

Agrawal, A. A. & Konno, K. (2009). Latex: A Model for Understanding Mechanisms, Ecology, and Evolution of Plant Defense Against Herbivory. *Annual Review of Ecology, Evolution, and Systematics*, *40* , 311–331. https://doi.org/10.1146/annurev.ecolsys.110308.120307

Appendino, G., Ech-Chahad, A., Minassi, A., De Petrocellis, L., Di Marzo, V. (2010). Structure–activity relationships of the ultrapotent vanilloid resiniferatoxin (RTX): The side chain benzylic methylene. *Bioorganic & Medicinal Chemistry Letters*, *20*(1), 97–99. https://doi.org/10.1016/j.bmcl.2009.11.035

Bellakhdar, J., Claisse, R., Fleurentin, J., Younos, C. (1991). Repertory of standard herbal drugs in the Moroccan pharmacopoea. *Journal of Ethnopharmacology*, *35*(2), 123–143. https://doi.org/10.1016/0378-8741(91)90064-K

Benabid, A. (2002). Flore et écosystèmes du Maroc Évaluation et préservation de la biodiversité (Ibis Press).

Bettar, I., González-Miret, M. L., Hernanz, D., Marconi, A., Heredia, F. J., Terrab, A. (2019). Characterisation of Moroccan Spurge (*Euphorbia*) honeys by their physicochemical characteristics, mineral contents and colour. *Arabian Journal of Chemistry*, *12*(8), 2052–2060. https://doi.org/10.1016/j.arabjc.2015.01.003

Bourhia, M., Shahat, A. A., Almarfadi, O.M., Naser, F.A., Abdelmageed, W.M., Ait Haj Said, A., El Gueddari, F., Naamane, A., Benbacer, L., Khlil, N. (2019). Ethnopharmacological survey of herbal remedies used for the treatment of cancer in the greater Casablanca-Morocco. *Evidence-Based Complementary and Alternative Medicine, 2019*, 1–9. https://doi.org/10.1155/2019/1613457

Bruyns P. V., Klak C., Hanacek P. (2011). Age and diversity in old world succulent species of *Euphorbia* (Euphorbiaceae). *Taxon*, *60*, 1717-1733. https://doi.org/10.1002/tax.606016

Davis S. D., Heywood V. H., Hamilton A. C. (1994). Centres Plant Divers. A Guide Strategy for their Conservation, v.3. Americas WWF IUCN, 20, 503–527.

Dorsey, B. L., Haevermans, T., Aubriot, X., Morawetz, J. J., Riina, R., Steinmann, V. W., Berry, P. E. (2013). Phylogenetics, morphological evolution, and classification of *Euphorbia* subgenus *Euphorbia*. *Taxon*, *62*(2), 291–315. https://doi.org/10.12705/622.1

Eggli,Urs. (2004). Illustrated Handbook of Succulent Plants: Dicotyledons. Springer Berlin, Heidelberg

Ettaqy, Abderrahim & Taha, Abdelhakim & ElGhiouane, Aziz & ElKhou, Anas & Abdelali, Boulli & Abbas, Younes. (2020). New data on the ecological distribution of *Euphorbia resinifera* O. Berg in the Beni Mellal-Khenifra region. E3S Web of Conferences, 183, 01001. https://doi.org/10.1051/e3sconf/202018301001

Farah, H. & Ech-chahad, A., Lamiri, A. (2014). In vitro antioxidant and antibacterial activity of the root extract of *Euphorbia resinifera*. *Journal of Pharmacognosy and Phytochemistry*, *2*(5), 161–163.

Fayed, A. A., Ahamed M. S., Faried A., Mohamed M. H. (2020). Leaf morphology and venation patterns of *Euphorbia* (Euphorbiaceae) in Egypt with special notes on their taxonomic implications. T*he Journal of Biological Sciences*, *13*(2), 165–176.

Fennane, M., & Ibn-Tattou, M. (2007). Flore pratique du Maroc: Manuel de détermination des plantes vasculaires Volume 2, Angiospermae (Leguminosae-Lentibulariaceae) (pp. 635). Institut Scientifique de rabat.

Govaerts, R., Frodin, D.G. & Radcliffe-Smith, A. (2000). World Checklist and Bibliography of Euphorbiaceae (and Pandaceae). Royal Botanic Gardens, Kew,1-4, 1-1622.

Hergenhahm, M., Adolf, W., Hecker, E. (1975). Resiniferatoxin and other esters of novel polyfunctional diterpenes from *Euphorbia resinifera* and *unispina*. Tetrahedron Letters, 19, 1595–1598.

Ihitassen A., Belrhazi Y., Bettar I., Msanda F. (2019). Melissopalynology and climatic variations: case of honey attributed to *Euphorbia resinifera*, region of Azilal (Morocco). I*nternational Journal of Advanced Research*, *7*(6), 702–725.

Jahandiez, É., Maire, R. (1932). Catalogue des Plantes du Maroc : (Spermaphytes et Ptéridophytes). Tome deuxième: Dicotylédones Archichlamydées 2 (pp 461). Alger.

Kabiri G., Bouda, S., Elhansali, M. & HAddioui, A. (2019). Genetic diversity and structure of walnut (Juglans regia L.) genotypes from Middle and High Atlas Mountains of Morocco as investigated by Inter-Simple Sequence Repeat (ISSR) markers. *Australian journal of crop science*, *13*(12),1983–1991.

Mahlberg, P. G., Pleszczynska, J., Rauh, W. (1983). Evolution of composition succulent as interpreted from latex. *Bothalia*,*14*(3/4), 857–863. https://doi.org/10.4102/abc.v14i3/4.1254

Mazoir, N., Benharref, A., Bailén, M., Reina, M., González-Coloma, A. (2008). Bioactive triterpene derivatives from latex of two *Euphorbia* species. *Phytochemistry*, *69*(6), 1328–1338. https://doi.org/10.1016/j.phytochem.2008.01.004

Mazoir, N., Benharref, A., Bailén, M., Reina, M., González-Coloma, A., Martínez-Díaz, R. A. (2011). Antileishmanial and Antitrypanosomal Activity of Triterpene Derivatives from Latex of Two *Euphorbia* Species. *Zeitschrift Für Naturforschung*, *C 66*(7–8), 360–366. https://doi.org/10.1515/znc-2011-7-807

MMA: Moroccan Ministry of Agriculture, Fisheries, Rural Development, Water and Forests. (2012). http://www.agriculture.gov.ma/en

MMA: Moroccan Ministry of Agriculture, Fisheries, Rural Development, Water and Forests. (2018). http://www.agriculture.gov.ma/sites/default/files/produitslabellises.pdf

Moujanni, A., Essamadi, A. K., Terrab, A. (2017). L'apiculture au {Maroc} : focus sur la production de miel. International Journal of Innovation and Applied Studies, 20 (1), 52–78. http://www.ijias.issr-journals.org/abstract.php?article=IJIAS- 16-313-03

Nemmaoui, A., Lorca, A. G., Aguilar, F. J., Aguilar M.A. (2013). Time series of vegetation for a destroyed forest model: The case of tadla azilal (Morocco). *Agrociencia*, *47*(3), 267–280.

Prenner G. & Rudall P. J. (2007). Comparative ontogeny of the cyathium in Euphorbia (Euphorbiaceae) and its allies: exploring the organ-flower-inflorescence boundary, *Amertica Journal of Botany*, *94*, 1612-1629

Pahlevani, A. H., Feulner, M., Weig, A., & Liede-Schumann, S. (2017). Molecular and morphological studies disentangle species complex in Euphorbia sect. Esula (Euphorbiaceae) from Iran, including two new species. *Plant Systematics and Evolution*, *303*(2), 139–164. https://doi.org/10.1007/s00606-016-1358-7

Sharma, P. K., Avula, B., Panda, A., Wang, Y. H., Khan, I. A., Murthy, S. N. (2019). A sensitive bioanalytical method for quantitative determination of resiniferatoxin in rat plasma using ultra-high performance liquid chromatography coupled to tandem mass spectrometry and its application in pharmacokinetic study. *Journal of Pharmaceutical and Biomedical Analysis*, *165*, 284–291. https://doi.org/10.1016/j.jpba.2018.12.024

Talbaoui, A. & Hamdaoui, L. (2020). Chemical Composition, in vitro Cytotoxic , and Antibacterial Activities of Moroccan Medicinal Plants *Euphorbia resinifera* and *Marrubium vulgare*. *Biointerface Research in Applied Chemistry*, *10*(6), 7343–7355. https://doi.org/10.33263/BRIAC106.73437355

Talebi, S. M., Noori M., Davijan S. S. (2016). Morphological study of some *Euphorbia* taxa in Iran. Nusantara Bioscience, 8 (1), 103–110

Wahid N, Martínez CG, EL Hadrami I, Boulli A .(2004). Genetic structure and variability of natural populations of Maritime Pine (Pinus pinaster Aiton) in Morocco. Silvae Genetica, 53 (3), 93–99

Zhang, K., Fan, W., Huang, Z., Chen, D., Yao, Z., Li, Y., Yang, Y., Qiu, D. (2019). Transcriptome analysis identifies novel responses and potential regulatory genes involved in 12-deoxyphorbol-13-phenylacetate biosynthesis of *Euphorbia resinifera*. *Industrial Crops and Products, 135(April)*, 138–145. https://doi.org/10.1016/j.indcrop.2019.04.030